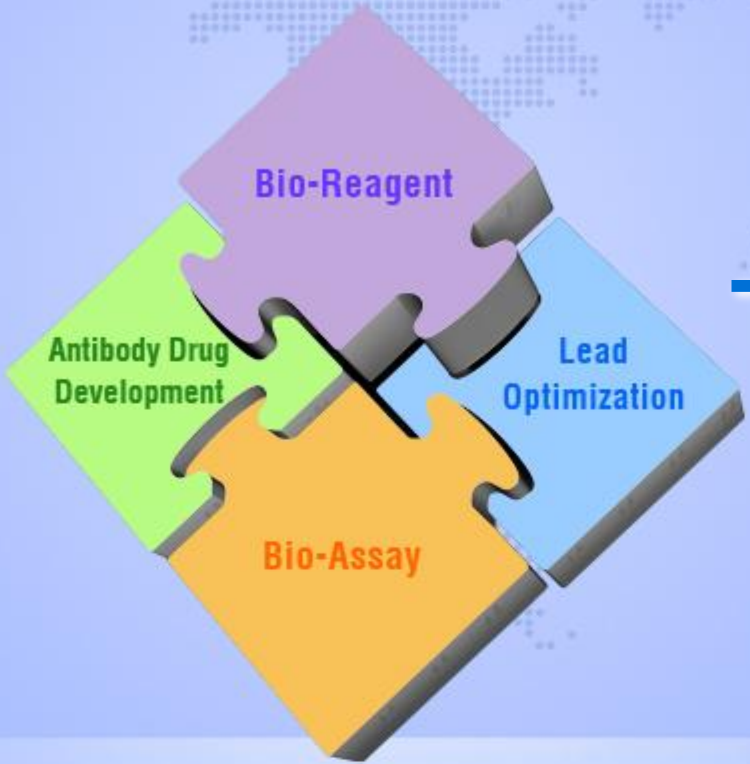
GenScript

The Biology CRO



Case Study

-- Library Construction

Your Innovation Partner in Drug Discovery!

Library construction

- Library diversity can be introduced with oligonucleotides, such as trimer-nucleotide or degenerated oligonucleotide.

- A codon design strategy is shown in the table below; this was from a case in which a mutant library was introduced for antibody optimization.

Wildtype		**Mutation design**
A (100%)	**M**	**A (70%); G (10%); C (10%); T (10%)**
T (100%)		**T (70%); A (10%); G (10%); C (10%)**
G (100%)		**G (70%); A (10%); C (10%); T (10%)**

Library construction

- The library was constructed according to the protocol of Kunkel’s method.

- We randomly sequenced 105 clones for quality control and the results are summarized as below.

Items	Number
Sequenced clones	105
Available clones with good signal	103
Deletion mutation clones	4
Insertion mutation clones	2
Correct clones	97
Correct insert rate	94.17%

Library construction

Wildtype		Distribution analysis (%)
G	V	G (74 / 76.29%); A (10 / 10.31%); T (8 / 8.25%); C (5 / 5.15%);
T		T (76 / 78.35%); G (8 / 8.25%); A (7 / 7.22%); C (6 / 6.19%);
G		G (73 / 75.26%); A (10 / 10.31%); T (10 / 10.31%); C (4 / 4.12%);
A	R	A (65 / 67.01%); T (14 / 14.43%); C (10 / 10.31%); G (8 / 8.25%);
G		G (62 / 63.92%); T (17 / 17.53%); A (10 / 10.31%); C (8 / 8.25%);
A		A (55 / 56.70%); G (19 / 19.59%); T (12 / 12.37%); C (11 / 11.34%);
C	Q	C (59 / 60.82%); G (15 / 15.46%); T (13 / 13.40%); A (10 / 10.31%);
A		A (64 / 66.67%); T (14 / 14.58%); G (11 / 11.46%); C (7 / 7.29%);
G		G (72 / 74.23%); T (11 / 11.34%); A (8 / 8.25%); C (6 / 6.19%);
A	M	A (49 / 50.52%); G (25 / 25.77%); C (13 / 13.40%); T (10 / 10.31%);
T		T (73 / 75.26%); A (13 / 13.40%); G (6 / 6.19%); C (5 / 5.15%);
G		G (73 / 75.26%); T (11 / 11.34%); C (8 / 8.25%); A (5 / 5.15%);
G	G	G (71 / 73.20%); T (12 / 12.37%); C (9 / 9.28%); A (5 / 5.15%);
G		G (74 / 76.29%); T (10 / 10.31%); A (8 / 8.25%); C (5 / 5.15%);
A		A (60 / 61.86%); C (15 / 15.46%); G (12 / 12.37%); T (10 / 10.31%);
C	Q	C (62 / 63.92%); G (18 / 18.56%); T (11 / 11.34%); A (6 / 6.19%);
A		A (54 / 55.67%); T (18 / 18.56%); G (15 / 15.46%); C (10 / 10.31%);
G		G (65 / 67.01%); C (13 / 13.40%); T (12 / 12.37%); A (7 / 7.22%);
G	G	G (68 / 70.10%); T (12 / 12.37%); C (12 / 12.37%); A (5 / 5.15%);
G		G (76 / 78.35%); C (9 / 9.28%); A (7 / 7.22%); T (5 / 5.15%);
C		C (71 / 73.20%); T (9 / 9.28%); G (9 / 9.28%); A (8 / 8.25%);
T	F	T (65 / 67.01%); G (14 / 14.43%); C (12 / 12.37%); A (6 / 6.19%);
T		T (70 / 72.16%); G (10 / 10.31%); C (10 / 10.31%); A (7 / 7.22%);
T		T (68 / 70.10%); G (12 / 12.37%); C (11 / 11.34%); A (6 / 6.19%);
G	D	G (72 / 74.23%); T (12 / 12.37%); C (7 / 7.22%); A (6 / 6.19%);
A		A (62 / 63.92%); T (13 / 13.40%); G (12 / 12.37%); C (10 / 10.31%);
T		T (73 / 75.26%); C (9 / 9.28%); A (8 / 8.25%); G (7 / 7.22%);
T	Y	T (82 / 84.54%); G (6 / 6.19%); C (5 / 5.15%); A (4 / 4.12%);
A		A (74 / 76.29%); G (10 / 10.31%); T (8 / 8.25%); C (5 / 5.15%);
C		C (65 / 67.01%); G (13 / 13.40%); T (10 / 10.31%); A (9 / 9.28%);

- We usually sequence at least 100 clones for library quality control.
- In this case, the sequencing results suggested that the mutant library was very well developed with high diversity, and the codon distribution was consistent with the initial design.